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Dr. Katrin Renz
University of New England, Armidale

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Researcher Contact Details
Dr. Katrin Renz
Animal Science W49
School of Environmental and Rural Science
University of new England
Armidale
NSW2351

Phone: 02 6773 3008
Fax: 02 6773 3922
Email:krenz@une.edu.au

In submitting this report, the researcher has agreed to the Australian Poultry CRC publishing this material in its edited form.

Australian Poultry CRC Contact Details
PO Box U242
University of New England
ARMIDALE NSW 2351

Phone: 02 6773 3767
Fax: 02 6773 3050
Email: info@poultrycrc.com.au
Website: http://www.poultrycrc.com.au

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Executive Summary

The aim of this project was to assist with filling the gap which has been left behind after the loss of the RMIT facilities and to implement routine cell culture work at UNE which will provide a source for cell culture skills and material for future avian research projects in Australia.
An audit of stock of isolates was undertaken to identify the origin of the various isolates held in Australia, how they are held (e.g. as titrated cell culture infectious material), their passage, history etc.

A range of MDVs currently held at UNE were grown on chicken embryo fibroblast (CEF) cell culture with the aim of growing to high titre ($\geq 10^4$ pfu/ml) so that they are available for challenge experiments. A total of nine isolates were chosen, amongst them MPF57, Woodlands1, 02LAR, FT158, and MPF23 a very virulent virus from the 1980s.

Protocols to propagate chicken embryo fibroblasts (CEF) for MDV were revised and optimized according to best international practice. For MDV we have decided to use CEF rather than chicken kidney (CK) cells because they are easy to grow and store, and the world reference laboratory for MD at IAH (Compton, UK) chooses to use them for all its work.

Several attempts to obtain monoclonal antibodies for immunostaining from USDA, East Lansing, Michigan, USA, to type and quantify the viruses in cell culture were unsuccessful. Therefore, plaques were enumerated at 6-7 days post inoculation under the microscope on duplicate 6-well titration plates. Cell-culture derived infective titrated material was then cryopreserved for long-term storage and future use.

DNA from cell cultured virus material was analysed in addition using quantitative PCR to correlate with plaque scores.

Contents

Introduction

Marek's disease has repeatedly demonstrated an ability to emerge from periods of adequate control to present a significant threat to industry – normally associated with failure of vaccinal control. MDV vaccines protect against the development of clinical MD, but not against infection with the virus. Upon exposure to MDV, vaccinated or unvaccinated chickens become carriers of the virus and persistently shed MDV into the environment. This has contributed to the evolution of MDV to more virulent isolates.
A number of virus isolation strategies are available; however, islolation of virus on cell culture is the preferred method to produce pure stocks of MDV isolates which can then be used in formal pathotyping or challenge experiments.

Recent research projects RIRDC UNE-83J and Poultry CRC 03-17 encountered an unexpected problem in the extreme difficulty in growing Australian MDV strains to moderate titre ($>10^4$pfu/ml) so that the isolates could be tested *in vivo*. Between 2002 and the end of 2005, from 655 promising isolates (i.e. screened as positive by a screening test) which were placed into cell culture, 17 infective isolates were recovered and only 4 isolates grew to a titre of 10^4 or higher (Walkden-Brown et al., 2006, Final Report RIRDC UNE 83-J). Isolates which previously grew to high titre were also difficult to grow again on further passage, or back-passage into chickens.

Earlier Australian researchers have also reported significant problems growing MDV to high titre (McKimm-Breschkin et al 1990; DeLaney et al 1995). On the other hand several groups around the world routinely grow field MDV isolates to titres of 10^6-10^9 pfu/ml.

The issue of failure of Australian MDVs to grow to high titre in cell culture in recent CRC and RIRDC projects remained unresolved and will be a problem for industry if/when MD again flares up.

In 2008, while Steve Walkden-Brown was on study leave at Dr Venugopal Nair's laboratory at IAH (Compton), Dr. Katrin Renz visited the institute and identified significant differences in methods used in those labs which suggest that the problem could be methodological, at least in part.

Thus, the purpose of this project was to deliver improved methodologies for isolation and propagation of Marek's disease in cell culture based on best international practice.

Objectives

- To audit the current stock materials of MDV isolates in Australia
- To grow one or more MDV isolates to a high titre ($>10^4$ pfu/ml) in CEF cell culture.
- To analyse DNA from cell cultured virus material for viral copy numbers in order to relate them with plaque counts.
- To cryopreserve cell-culture derived infective and titrated material for long-term storage and future use.

Methodology

Laboratories

All work was performed in the poultry virology and molecular biology laboratories at UNE which are equipped with CO_2 incubators, biohazard and laminar flow cabinets, inverted microscopes together with PCR and real-time PCR facilities. Confocal and electron microscopy facilities are also available. The virology lab was reactivated by this project after being dormant for some years due to lack of a

microbiologist to run it. The laboratory previously had OGTR and microbiological PC2 status, and this was re-instated as part of the project to enable future work with GMO and pathogens.

MDV isolation on cell culture including virus handling and storage

This short project focused on isolation and growth of MDV isolates to high titre in CEF cultures.

Media

The media used to prepare and propagate CEF cultures was M199 (Invitrogen Australia Pty Ltd.) and prepared according to manufacturer's instructions. The media was then sterile filtrated into sterile Schott bottles and stored at 4◦C.

For M199 growth media, 10% foetal calf serum, heat-inactivated (Invitrogen Australia Pty Ltd.) and 5% Antibiotic/Antimycotic containing penicillin, streptomycin and amphotericin (Invitrogen Australia Pty Ltd.) was added. 5% foetal calf serum as added for M199 maintenance media. For cryopreservation, 10% cell culture grade DMSO (Sigma) was added M199 growth media.

PBS was prepared from tablets (Sigma) according to manufacturer's instructions and autoclaved.

Activated sterile 0.05% trypsin/versene solution (Invitrogen Australia Pty Ltd.) was used to split CEF cell layers and to passage the virus.

Preparation of avian fibroblasts

- Fertile SPF eggs were obtained from SPAFAS Australia (ex CSIRO HWL line) and placed in an incubator set at 38.5ºC for 9-11 days.
- Eggs were candled at 7-8 days of incubation to check the viability of the embryos.
- Two eggs at a time were cleaned with 70% ethanol using a sterile cotton wool pad.
- The blunt end was broken and cut open with sterile scissors and, the embryo removed into a sterile petri-dish containing pre-warmed sterile PBS and the head was immediately removed to kill the embryos.
- Any blood and intestines were removed and the remaining embryo finely chopped with scissors.
- Using a sterile 10 ml syringe, the embryos were then transferred into a sterile 250ml conical flask containing a magnetic stirrer.
- Approximately 25 ml pre-warmed PBS were added to the embryos and vigorously shaken to wash off red blood cells. The contents were allowed to settle and the supernatant was then discarded. This step was repeated twice.
- 20 ml of warm trypsin/versene solution was added to the conical flask and agitated for approximately 2-3 min. The contents were allowed to settle before pouring off the cell suspension into a sterile centrifuge tube containing 2 ml pre-warmed foetal calf serum. This step was repeated three times.
- The tubes containing the cell suspension were then centrifuged at 450xg for 5 min at 5ºC to pellet the cells.
- The supernatant was poured off and cells from all tubes were resuspended in 20 ml warm growth medium.
- The cell suspension was filtered through a 40 µm cell sieve into a sterile 50 ml centrifuge tube.
- Cells were then counted using a haemocytometer, adjusted to $2x10^7$cells/ml and either prepared for cryopreservation with M199 growth media containing 10% DMSO or immediate use in cell culture flasks. Flasks with 25cm^2 area (Greiner, Germany) were seeded with approx. $2x10^6$ cells. Flasks with 75 cm^2 area (Greiner, Germany) were seeded with approx. $8x10^6$ cells.
- Primary CEFs were grown overnight in a CO_2 incubator at 37.5ºC and 5% CO_2 to 95-100% confluence and split 1:2 on the following day to produce secondary CEFs.

- Secondary CEFs were used to infect with MDV isolates 24 hours after seeding/splitting and media changed every alternate day until 7 dpi.

Infectious materials

Where possible, cryopreserved splenocytes from unvaccinated challenged SPF animals were used to infect secondary CEF cultures. Alternatively, low passage cell cultured material was used to grow MDV isolates to high titre. Table 1 shows the details of the materials used in this project.

Table 1: MDV isolates used in CEF cultures.

Original isolate name	Alias name	Material to infect CEF	Batch no.	Passage no.	Titre (Pfu/ml)
Woodlands1		Fresh splenocytes	131009		
02LAR	210/2s	Cryopreserved splenocytes	MD05-R-PT3		
		cell culture material	020406	6	30
FT158	210/1s	Low passage cell culture material	020406	6	17
MPF23		Cryopreserved splenocytes and whole blood	080805		
MPF57	179/6	cell cultured material	020406	6	300
MPF164/6		Cryopreserved splenocytes	MD05-R-PT3		
MPF189/8		Cryopreserved splenocytes	MD05-C-VI5		
MPF192		Cryopreserved splenocytes	MD05-C-VI5		
MPF199/3&9		Cryopreserved splenocytes	MD05-C-VI5		
		cell cultured material	020406	4	200

Passage of virus

- At 6-7 dpi, the virus isolates were passaged onto new secondary CEF.
- Cell layers were trypsinized with 3 ml pre-warmed trypsin/versene mix and incubated at 37.5ºC and 5% CO_2 for approximately 3 min to detach the cells from the flask.
- The cells were then resuspended in 4 ml warm foetal or newborn calf serum and centrifuged at 450xg for 5min at 5ºC to pellet the cells.
- The cells were then resuspended in 4 ml warm M199 growth media and 3.5 ml of the suspension added to new confluent secondary CEFs grown overnight. The remaining 0.5 ml was stored at -20ºC for DNA extraction and PCR analysis.

Titration of virus

- To titrate virus material, 6-well plates were seeded with secondary CEFs @ 1.2 x10^6 cells per well. 100 ul of virus material to be titrated was taken from the virus passage and 10-fold serial dilutions prepared. 200 ul of each dilution, ranging from 10^0 – 10^{-5} were added to one well and incubated until 7 dpi.
- Plaques were counted under an inverted microscope at the dilution which gave the easiest distinction between plaques (usually 10^{-3}) and the titre calculated as follows:

  Count x 5 x dilution factor = titre (pfu/ml)

Cryopreservation of virus

- After titration of the virus material, the cell layers were trypsinized with 3 ml pre-warmed trypsin/versene mix and incubated at 37.5ºC and 5% CO_2 for approximately 3 min to detach the cells from the flask.
- The cells are then resuspended in freezing medium (M199 growth medium with 10% DMSO). A 75cm^2 flask should be resuspended in approximately 5ml freezing medium.
- The cell/ virus suspension is then aliquoted into 1ml cryovials (Greiner, Germany) and placed in a 'Mr. Frosty' freezing jar in -80ºC overnight before being transferred into liquid nitrogen.

PCR analysis of cell cultured material

The remainder of each virus passage material as well as the original materials used were stored at -20°C prior to DNA extraction using the QIAGEN kit (Qiagen, Clifton Hill, Australia) according to manufacturer's instructions. Extracted DNA was quantified using a NanoDrop® ND-1000 UV-Vis spectrophotometer (NanoDrop® Technologies Wilmington, USA) before performing real-time PCR assays as described previously by Islam et al. (2004; 2006).

Results

Audit of MDV isolates

An audit of MDV materials stored at various locations in Australia was performed. Table 1 below lists both cell cultured and non cell cultures materials. Most cell cultured infectious materials are currently stored at UNE in Armidale. A DNA library containing numerous international MDV isolates is held at James Cook University in Townsville. The details of the audit are listed in the Appendix.

CEF cultures

CEF cultures were successfully prepared from 9-11 day old embryos and grew to 90-100 % confluence within 24-36 hours (Plate 1). In order to determine the longevity of the cells, one flask was kept uninfected and was incubated for 14 days with media changes on each second day. The cultures did not deteriorate between 7 and 10 days, but thereafter, they showed signs of 'ageing', i.e. shrinking and some cells started to come off.

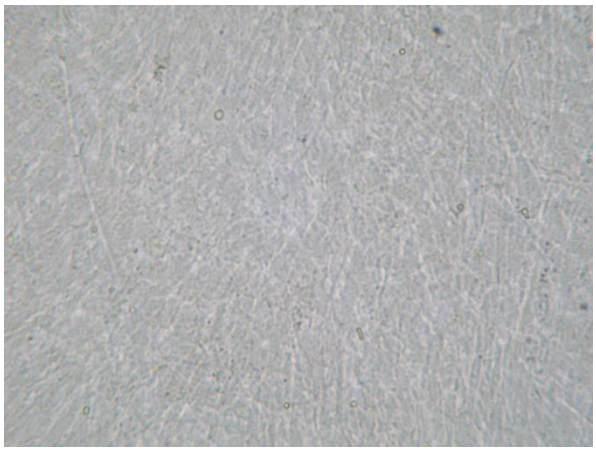


Plate 1: Phase contrast image of uninfected CEF, 24 hours after seeding, magnification 5:1.

Pathogenic MDV isolates in CEF

Five of the nine selected MDV isolates were successfully grown to high titre (>10^4 pfu/ml) within 3-4 passages in CEF (Table 2). Phase contrast images of plaques were taken between 5-7 dpi from each of the 5 isolates (Plate 2).

The remaining 4 isolates did not show plaques after 3 passages in CEF and were discarded. In the case of the isolate MPFF199/3&9, it was obvious that the cryopreserved materials used to infect freshly prepared confluent CEF cultures were contaminated. The CEF cells detached from the culture flask within 24 hours after infection.

Table 2: MDV isolates which successfully grew to high titre (≥10^4 pfu/ml).

Isolate name	Batch no.	Passage no.	Pfu/ml	Volume cryopreserved	$Log_{10}VCN/10^6$ cells
Woodlands1	041109	3	90,000	7ml	9.34
02LAR	181109	4	155,000	14ml	8.77
FT158	021209	4	165,000	7ml	8.87
MPF23	021209	4	80,000	7ml	8.87
MPF57	181109	4	60,000	12ml	8.84

Figure 1 shows the increase of virus titre between passages for all 5 MDV isolates. The isolate Woodlands1 grew from 6.15 log_{10} $VCN/10^6$ cells to 9.34 log_{10} $VCN/10^6$ cells at passage 3 at which stage this isolate was cryopreserved. The remaining 4 isolates grew from 6.26-7.78 log_{10} $VCN/10^6$ cells to 8.84-8.76 log_{10} $VCN/10^6$ cells at passage 4 at which stage all these isolates were cryopreserved. Generally, the viral copy number did increase exponentially between passage 1 and 2 or 2 and 3 and then continued to increase in a linear manner up to passage 4 with the exception of the isolate 02LAR which plateaued between passage 2 and 3 and decreased between passage 3 and 4. (Figure1).

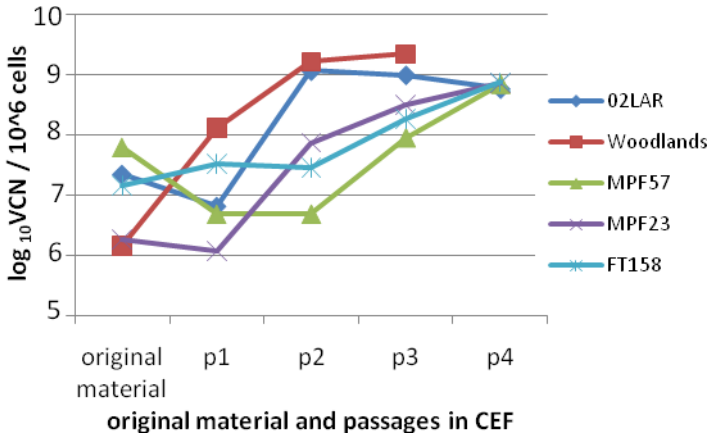
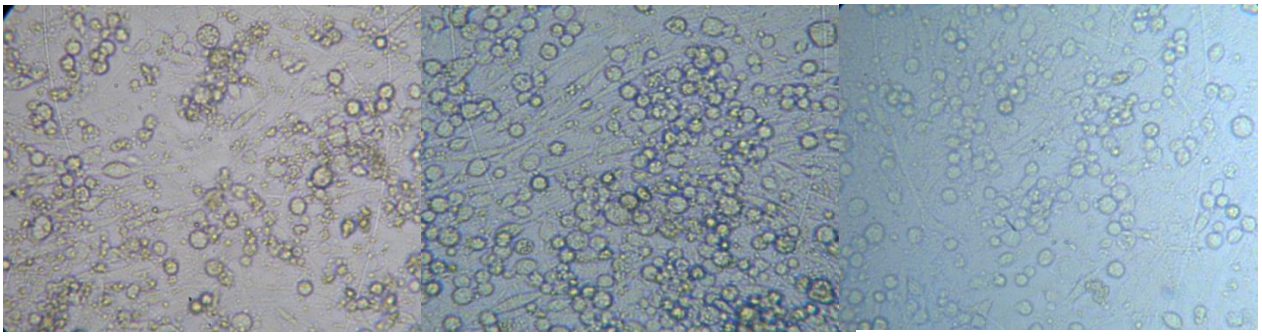
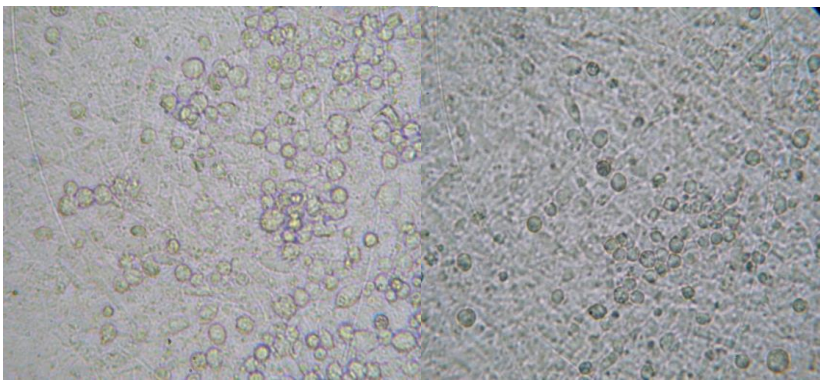


Figure 1: Increase of virus titre between passages.

Plate 2 shows phase contrast images of each isolate using a magnification of either 10:1 or 5:1. Plaques were usually present after 4-5 dpi and were enumerated at 7 dpi on each passage.



Woodlands1 02LAR FT158



MPF57 MPF23

Plate 2: Phase contrast images of MDV isolates which grew to high titre ($\geq 10^4$ Pfu/ml) in CEF, magnification 10:1/5:1.

Discussion

This short-term strategic project aimed at optimising the isolation and propagation methods for MDV isolates. Five MDV isolates grew successfully to high titres ($\geq 10^4$ pfu/ml), which previously was a major issue and threatened the execution of the previous CRC project 03-17 (Walkden-Brown et al., 2007).

The major differences to the protocols previously used in Australia in order to isolate and propagate MDV isolates (Tan et al., 2008) were the use of M199 media and higher amounts of foetal bovine serum. The M199 medium is a complete medium containing a complete range of essential amino acids and salts as well as adenosin monophosphate (AMP) and adenosine triphosphate (ATP) (Morgan et al., 1950). In contrast, the MEM medium only contains a reduced range of those ingredients without AMP and ATP and in earlier studies at UNE, M199 has been used in order to grow the serotype 3 vaccinal isolate HVT (Islam et al., 2001). Foetal calf serum contains a wide variety of proteins and growth factors which stimulate cell growth and proliferation. To prepare growth and maintenance media, 10% and 5% foetal calf serum were used for this project which is double the amount than what has been used in previous studies in Australia (Tan et al., 2008).

In contrast to earlier problems encountered in Australia when attempting to grow MDV isolates and increase their titre to 10^4 pfu/ml or above in chicken kidney cells (Walkden-Brown et al., 2007), this project has demonstrated that titres can be significantly increased through passaging them on 3-4 times on CEF cultures. This is contrary to previous work which found that chicken embryo fibroblasts for isolation and propagation of MDV isolates has previously be shown to be inferior in terms of first appearance of plaques, virus yield and virus increase after passaging (Schat, 2005). However, the international reference laboratory for Marek's disease in Compton, UK, routinely and successfully uses CEF cells for isolation and propagation as they are more robust and easier to handle than chicken kidney cells. The previous study reported by Walkden-Brown et al. (2007) initially also used CEF cells before changing over the chicken kidney cells in 2004 due to higher virus yield in chicken kidney cells. However, the lower virus yield in CEF cultures might have been due to the use of a different, less complete media as mentioned above. It is likely that this had an effect on the growth of the virus isolates resulting in a lower yield in CEF cultures. This suggests that both the cell line and the cell culture medium play an equally important part in order to maximize successful isolation and propagation of MDV.

However, this project did not investigate whether Australian MDV isolates have certain biological properties which prevent or impede isolation and adaptation to cell culture systems. There are indications that some overseas MDV isolates of increased virulence are more difficult to adapt and grow in cell culture. However, the previous CRC project 03-17 identified Australian MDV isolates to be in the v or vv pathotype category according to the USDA ADOL method (Witter et al., 2005), but with little evidence that isolates of increased virulence circulate in Australia. More research should be undertaken to resolve this issue as it poses a threat for future research on Marek's disease in Australia.
The virus isolates which were retrieved from cryopreserved materials grew in an exponential manner after the second passage. Viral copy numbers (VCN) per 10^6 cells as determined by qPCR analysis dropped during the first passage compared to the original material. This was expected as a certain amount of cells and virus are usually not viable after the freeze/ thaw cycle from liquid nitrogen. In contrast, virus from freshly prepared splenocytes (Woodlands1) grew in a steep linear manner from passage 1 onwards and reached high titres within 3 passages whereas all other viruses had to be passaged once more. This suggests that fresh infective material should be used wherever possible in order to produce high titres in cell culture within the lowest possible passages.

Interestingly, plaque counts of the end point virus titrations were not consistent with qPCR results. This may be explained due to the fact that qPCR will detect both live and dead virus as well as virus fractions as long as it finds the matching DNA template in the sample to be analysed whereas the and enumeration of plaques on cell culture only represents live virus. However, monoclonal antibodies are recommended to use for the staining of plaques for future work in order to ascertain that only MDV specific plaques are counted.

Implications

This project delivered improved methodologies for MDV isolation and propagation in cell culture based on best international practice. This significantly improves the capacity for the Australian poultry industry to manage one of its most important endemic diseases. The work of this project could serve as a template for future isolation and propagation of MDV in cell culture.

The major implications of this project are:

1. Australian MDV isolates can be grown in cell culture to high titres ($\geq 10^4$ pfu/ml) within low passage numbers (3-4), especially when fresh materials, i.e. splenocytes are used.
2. The cell culture media, serum and cell type significantly influences the ability to successfully isolate and propagate MDV isolates. Chicken embryo fibroblasts are easier to prepare, handle and grow and were therefore chosen for all work done in this project. This is in accordance with the IAH institute in Compton, UK, which is the international reference laboratory for Marek's disease.
3. Results from qPCR are not necessarily consistant with plaque counts. The reason might be that PCR will amplify both dead, damaged and live virus particles whereas visible plaques in cell culture only represent live virus.

Recommendations

This project has not produced a product of direct commercial value. However, it has delivered valuable tools which can be used in the future to isolate and propagate MDV in cell culture. This will significantly improve the capacity for the Australian poultry industry to manage one of its most important endemic diseases.
The major recommendations arising from this work are as follows:

1. MDV isolates should be isolated on CEF cultures rather than CKC.
2. M199 media and 5-10% foetal calf serum should be used to prepare growth and maintenance media.
3. For isolates which grow poorly, further work should be done to resolve the issue of whether isolation and growth on cell culture, even with these improved methods, is due to biological variation between MDV isolates as there is some evidence that international MDV isolates of greater virulence are more difficult to grow or visualise in cell culture.
4. Consideration should be given to amplify the virus in SPF chickens and before attempting to isolate virus from fresh splenocytes.

Appendix

1. List of cell cultured materials

original isolate name	alias name	batch no.	passage no.	cells used to grow it	cell culture media	titre (pfu/ml)	amount of material held (ml)	storage method	storage location	number of backpassages in chickens since original isolate	year of original isolation	place of origin	flock of origin	MD vaccination history
MPF 57		011103	12	CEF	MEM	unknown	16.2	liqN	UNE	0	1994	NSW Sydney	14wo layers	unknown
MPF 57		100703	12	CEF	MEM	unknown	10.8	liqN	UNE	0	1994	NSW Sydney	14wo layers	unknown
MPF 57		141003	12	CEF	MEM	unknown	3.6	liqN	UNE	0	1994	NSW Sydney	14wo layers	unknown
MPF 57		230603	12	CEF	MEM	unknown	1.8	liqN	UNE	0	1994	NSW Sydney	14wo layers	unknown
MPF 57		230603	12	CEF	MEM	unknown	9	liqN	UNE	0	1994	NSW Sydney	14wo layers	unknown
MPF 57		070703	12	CEF	MEM	unknown	30.6	liqN	UNE	0	1994	NSW Sydney	14wo layers	unknown
MPF 57		020406	4	CK	MEM	unknown	3.6	liqN	UNE	3	1994	NSW Sydney	14wo layers	unknown
MPF 176	MPF57 B1	020406	6	CK	MEM	27	3.6	liqN	UNE	1	1994	NSW Sydney	14wo layers	unknown
MPF 176	MPF57 B1	030504	4	CK	MEM	unknown	4.8	liqN	UNE	1	1994	NSW Sydney	14wo layers	unknown
MPF 179/6	MPF57 B1	020406	6	CK	MEM	300	34.2	liqN	UNE	2	1994	NSW Sydney	14wo layers	unknown
MPF 179/6	MPF57 B1	200904	7	CK	MEM	unknown	7.2	liqN	UNE	1	1994	NSW Sydney	14wo layers	unknown
MPF 163/10		100703	5	CEF	MEM	3302	3.6	liqN	UNE	unknown				
MPF 179/2	04CRE	260904	8	CK	MEM	26000	1.8	liqN	UNE	unknown	2004	NSW Sydney Victoria	6wo layers	Rispens
MPF 179/3	02LAR	120904	6	CK	MEM	9833	0	liqN	UNE	unknown	2002	Mornington P	broilers	unknown
MPF 179/8	MPF 132/5	050904	5	CK	MEM	147000	19.8	liqN	UNE	unknown	2001	NSW	broilers	unknown
MPF 189/8		020406	6	CK	MEM	60	3.6	liqN	UNE	1	2004	QLD	broilers	unvaccinated
MPF 192/1		190705	12	CK	MEM	unknown	1.8	liqN	UNE	unknown	2004	SA	broilers	unvaccinated
MPF 192/4&10		020406	6	CK	MEM	140	16.2	liqN	UNE	1	2004	SA	broilers	unvaccinated
MPF 199/3&9		020406	4	CK	MEM	200	1.8	liqN	UNE	2	2004	SA	broilers	unvaccinated
MPF 210/1s	FT158 B1	020406	6	CK	MEM	100	1.8	liqN	UNE	2	2002	northern NSW Victoria	broiler breeder	Rispens
MPF 210/2s	02LAR B1	020406	4	CK	MEM	70	3.6	liqN	UNE	3	2002	Mornington P Victoria	broilers	unknown
MPF 210/2s	02LAR B1	020406	6	CK	MEM	30	1.8	liqN	UNE	2	2002	Mornington P	broilers	unknown
MPF 212	05JUR B1	020406	6	CK	MEM	210	10.8	liqN	UNE	2	2005	NSW Sydney	77wo layers	Rispens
MPF23		190705	3	CK	MEM	unknown	7.2	liqN	UNE	2?	mid 1980's	Victoria	unknown	unknown
MPF23		020406	4	CK	MEM	60	1.8	liqN	UNE	3?	mid 1980's	Victoria	unknown	unknown

original isolate name	alias name	batch no.	passage no.	cells used to grow it	cell culture media	titre (pfu/ml)	amount of material held (ml)	storage method	storage location	number of backpassages in chickens since original isolate	year of original isolation	place of origin	flock of origin	MD vaccination history
05JUR					MEM	unknown		liqN	UNE	1	2005	NSW Sydney	77wo layers	Rispens
W7B1S	MPF57 B2	020406	6	CK	MEM	110	3.6	liqN	UNE	2	1994	NSW Sydney	14wo layers	unknown
Woodlands 1		310804	14	CK	MEM	unknown	1.8	liqN	UNE	unknown	1992	SE QLD	broiler breeder	Bivalent (sero 2&3)
Woodlands 1		051109	2	CEF	M199	unknown	5	liqN	UNE	1?	1992	SE QLD	broiler breeder	bivalent (sero 2&3)
WDS	Woodlands 1	020406	4	CK	MEM	600	3.6	liqN	UNE	3	1992	SE QLD	broiler breeder	bivalent (sero 2&3)
WDS	Woodlands 1	020406	6	CK	MEM	680	16.2	liqN	UNE	2	1992	SE QLD	broiler breeder	Bivalent (sero 2&3)
FT158		020406	6	CK	MEM	17	1.8	liqN	UNE	1	2002	northern NSW	broiler breeder	Rispens
04KAL		020406	4	CK	MEM	140	14.4	liqN	UNE	3	2004	SA		unvaccinated
02LAR		020406	6	CK	MEM	60	1.8	liqN	UNE	2	2002	Victoria Mornington P	broilers	unknown
MPF 155		050603	4	CEF	MEM	unknown	5.4	liqN	UNE	unknown				
MPF 177		120504	7	CK	MEM	unknown	5.4	liqN	UNE	unknown				
Woodlands CSIRO			1	CEF	M199	unknown	1	liqN	Richie Laboratories, UQ	unknown				
Woodlands RMIT			7	CEF	M199	unknown	4	liqN	Richie Laboratories, UQ	unknown				
192			6	CK	unknown	140	1.5	liqN	Richie Laboratories, UQ	unknown		SA		
192			12	CK	unknown	1250	1.5	liqN	Richie Laboratories, UQ	unknown		SA		
MPF57 B1	179/6		7	CK	unknown	20000	1.5	liqN	Richie Laboratories, UQ	unknown		NSW		

2. List of non- cell cultured materials

original isolate name	alias name	batch	form the material is held in	amount of material held (ml)	storage method	storage location	number of backpassages in chickens since original isolate	year of original isolation	place of origin	flock of origin	MD vaccination history
MPF 199/3&9		270206	splenocytes	5.4	liqN	UNE	2	2004	SA	broilers	unvaccinated
MPF 189/8		270206	splenocytes	9	liqN	UNE	2	2004	QLD	broilers	unvaccinated
04KAL		270206	splenocytes	7.2	liqN	UNE	2	2004	SA		unvaccinated
MPF 210/1s	FT158 B1	270206	splenocytes	9	liqN	UNE	2	2002	northern NSW	broiler breeder	Rispens
W7B1S	MPF57 B2	141205	splenocytes	32.4	liqN	UNE	2	1994	NSW Sydney	14wo layers	unknown
MPF 210/2s	02LAR B1	141205	splenocytes	9	liqN	UNE	2	2002	Victoria Mornington P	broilers	unknown
MPF164/6		141205	splenocytes	18	liqN	UNE	1				
MPF 179/6	MPF57 B1	141205	splenocytes	12.6	liqN	UNE	2	1994	NSW Sydney	14wo layers	unknown
Woodlands 1		141205	splenocytes	19.8	liqN	UNE	1	1992	SE QLD	broiler breeder	bivalent (sero 2&3)
MPF 192/1		141205	splenocytes	12.6	liqN	UNE	1	2004	SA	broilers	unvaccinated
02LAR		141205	splenocytes	63	liqN	UNE	2	2002	Victoria Mornington P	broilers	unknown
MPF23		180705	whole blood	9	liqN	UNE	unknown	mid 1980's	Victoria	unknown	unknown
MPF23		80805	whole blood	82.8	liqN	UNE	unknown	mid 1980's	Victoria	unknown	unknown
MPF23		80805	splenocytes	7.2	liqN	UNE	unknown	mid 1980's	Victoria	unknown	unknown
MPF23		140905	splenocytes	1.8	liqN	UNE	unknown	mid 1980's	Victoria	unknown	unknown
Woodlands 1		131009	splenocytes	3.6	liqN	UNE	3	1992	SE QLD	broiler breeder	bivalent (sero 2&3)
Woodlands 1		131009	whole blood	5.4	liqN	UNE	3	1992	SE QLD	broiler breeder	bivalent (sero 2&3)
MPF207		P4 180105	splenocytes	1.8	liqN	UNE	unknown				
W7B1S	MPF57 B2	141205	whole blood	3.6	liqN	UNE	1	1994	NSW Sydney	14wo layers	unknown
MPF 199/3&9		141205	whole blood	5.4	liqN	UNE	1	2004	SA	broilers	unvaccinated
MPF 57		141205	whole blood	5.4	liqN	UNE	1	1994	NSW Sydney	14wo layers	unknown
MPF 176/734o,734s, 94		141205	whole blood	3.6	liqN	UNE	1	1994	NSW Sydney	14wo layers	unknown

original isolate name	alias name	batch	form the material is held in	amount of material held (ml)	storage method	storage location	number of backpassages in chickens since original isolate	year of original isolation	place of origin	flock of origin	MD vaccination history
MPF 210/2s	02LAR B1	141205	whole blood	3.6	liqN	UNE	1	2002	Victoria Mornington P	broilers	unknown
MPF164/6		141205	whole blood	1.2	liqN	UNE	1				
MPF 212	05JUR B1	141205	whole blood	3.6	liqN	UNE	2	2005	NSW Sydney	77wo layers broiler	Rispens
MPF 210/1s	FT158 B1	141205	whole blood	3.6	liqN	UNE	2	2002	northern NSW	breeder	Rispens
MPF 179/6	MPF57 B1	141205	whole blood	3.9	liqN	UNE	2	1994	NSW Sydney	14wo layers	unknown
04OWE		141205	whole blood	1.8	liqN	UNE	1	2004	SA		unvaccinated
MPF 189/8		141205	whole blood	4.8	liqN	UNE	1	2004	QLD	broilers	unvaccinated
MPF23 (100ul)		141205	whole blood	1.2	liqN	UNE	1	mid 1980's	Victoria	unknown broiler	unknown
Woodlands 1		141205	whole blood	7.2	liqN	UNE	1	1992	SE QLD	breeder	bivalent (sero 2&3)
MPF 192/4&10		141205	whole blood	3.6	liqN	UNE	1	2004	SA	broilers	unvaccinated
MPF 192/1		141205	whole blood	3.6	liqN	UNE	1	2004	SA	broilers	unvaccinated
02LAR		141205	whole blood	5.4	liqN	UNE	1	2002	Victoria Mornington P	broilers	unknown
04KAL		141205	whole blood	3.6	liqN	UNE	1	2004	SA		unvaccinated

3. List of DNA material

original isolate name	alias name	amount of material held (ml)	storage method	storage location	Origin	DNA details	comments
HPRS-B14		4ul	TE buffer	UNE	IAH Compton, UK	whole genome	vMDV1
JM102/W		4ul	TE buffer	UNE	IAH Compton, UK	whole genome	vMDV1
571		4ul	TE buffer	UNE	IAH Compton, UK	whole genome	vMDV1
RB-1B		4ul	TE buffer	UNE	IAH Compton, UK	whole genome	vvMDV1
Md5		4ul	TE buffer	UNE	IAH Compton, UK	whole genome	vvMDV1
549A		4ul	TE buffer	UNE	IAH Compton, UK	whole genome	vvMDV1
595		4ul	TE buffer	UNE	IAH Compton, UK	whole genome	vvMDV1
584A		4ul	TE buffer	UNE	IAH Compton, UK	whole genome	vv+MDV1
648A		4ul	TE buffer	UNE	IAH Compton, UK	whole genome	vv+MDV1
660A		4ul	TE buffer	UNE	IAH Compton, UK	whole genome	vv+MDV1
675A		4ul	TE buffer	UNE	IAH Compton, UK	whole genome	vv+MDV1
MR22			ethanol precipitated/ dehydrated	JCU	Intervet (Boxmeer, Holland)	whole genome	
MR36			ethanol precipitated/ dehydrated	JCU	Intervet (Boxmeer, Holland)	whole genome	
MR48			ethanol precipitated/ dehydrated	JCU	Intervet (Boxmeer, Holland)	whole genome	
HPRS16			ethanol precipitated/ dehydrated	JCU	Intervet (Boxmeer, Holland)	whole genome	mMDV
CU-2 (p15)			ethanol precipitated/ dehydrated	JCU	Cornell University (Ithaca, NY)	whole genome	
CVI988 (p49)			ethanol precipitated/ dehydrated	JCU	Cornell University (Ithaca, NY)	whole genome	

original isolate name	alias name	amount of material held (ml)	storage method	storage location	Origin	DNA details	comments
GA-5 (p19)			ethanol precipitated/ dehydrated	JCU	Cornell University (Ithaca, NY)	whole genome	mMDV
JM-10 (p12)			ethanol precipitated/ dehydrated	JCU	Cornell University (Ithaca, NY)	whole genome	
JM-16 (p19)			ethanol precipitated/ dehydrated	JCU	Cornell University (Ithaca, NY)	whole genome	
JM-16 (p43)			ethanol precipitated/ dehydrated	JCU	Cornell University (Ithaca, NY)	whole genome	
JM-16 (p71)			ethanol precipitated/ dehydrated	JCU	Cornell University (Ithaca, NY)	whole genome	
Md11 (p16)			ethanol precipitated/ dehydrated	JCU	Cornell University (Ithaca, NY)	whole genome	
R2/23 (p119)			ethanol precipitated/ dehydrated	JCU	Cornell University (Ithaca, NY)	whole genome	
Md5 (p12)			ethanol precipitated/ dehydrated	JCU	Cornell University (Ithaca, NY)	whole genome	vvMDV
RB-1B (p38)			ethanol precipitated/ dehydrated	JCU	Cornell University (Ithaca, NY)	whole genome	vvMDV1
Rb-1B (p72)			ethanol precipitated/ dehydrated	JCU	Cornell University (Ithaca, NY)	whole genome	vvMDV1
RK-1 (p15			ethanol precipitated/ dehydrated	JCU	Cornell University (Ithaca, NY)	whole genome	
HVT (p32)			ethanol precipitated/ dehydrated	JCU	Cornell University (Ithaca, NY)	whole genome	
SB-1 (p9)			ethanol precipitated/ dehydrated	JCU	Cornell University (Ithaca, NY)	whole genome	

original isolate name	alias name	amount of material held (ml)	storage method	storage location	Origin	DNA details	comments
Md5			ethanol precipitated/ dehydrated	JCU	University of Arkansas (Fayetteville, Ark)	whole genome	vvMDV
571			ethanol precipitated/ dehydrated	JCU	University of Arkansas (Fayetteville, Ark)	whole genome	vMDV
549			ethanol precipitated/ dehydrated	JCU	University of Arkansas (Fayetteville, Ark)	whole genome	vvMDV
643p			ethanol precipitated/ dehydrated	JCU	University of Arkansas (Fayetteville, Ark)	whole genome	vvMDV
BC-1			ethanol precipitated/ dehydrated	JCU	University of Arkansas (Fayetteville, Ark)	whole genome	vMDV
RB-1B			ethanol precipitated/ dehydrated	JCU	University of Arkansas (Fayetteville, Ark)	whole genome	vvMDV1
JM-10 (CU210 cells)			ethanol precipitated/ dehydrated	JCU	University of Arkansas (Fayetteville, Ark)	whole genome	vMDV
CU-2			ethanol precipitated/ dehydrated	JCU	University of Arkansas (Fayetteville, Ark)	whole genome	mMDV
L-strain			ethanol precipitated/ dehydrated	JCU	University of Arkansas (Fayetteville, Ark)	whole genome	vv+MDV1
RL-strain			ethanol precipitated/ dehydrated	JCU	University of Arkansas (Fayetteville, Ark)	whole genome	vv+MDV1
686			ethanol precipitated/ dehydrated	JCU	University of Arkansas (Fayetteville, Ark)	whole genome	vv+MDV1
New strain			ethanol precipitated/ dehydrated	JCU	University of Arkansas (Fayetteville, Ark)	whole genome	vv+MDV1
595			ethanol precipitated/ dehydrated	JCU	University of Arkansas (Fayetteville, Ark)	whole genome	vv+MDV1

original isolate name	alias name	amount of material held (ml)	storage method	storage location	Origin	DNA details	comments
660-A			ethanol precipitated/ dehydrated	JCU	University of Arkansas (Fayetteville, Ark)	whole genome	vv+MDV1
648-A			ethanol precipitated/ dehydrated	JCU	University of Arkansas (Fayetteville, Ark)	whole genome	vv+MDV1
TK strain			ethanol precipitated/ dehydrated	JCU	University of Arkansas (Fayetteville, Ark)	whole genome	vv+MDV1
			plasmid	JCU			
JC5-001-a	MPF 57 P9 300801		plasmid	JCU		vIL-8 Prom	
JC5-001-b	MPF 57 P9 300801		plasmid	JCU		vIL-8 Prom	
JC5-001-c	MPF 57 P9 300801		plasmid	JCU		vIL-8 Prom	
JC5-001-d	MPF 57 P9 300801		plasmid	JCU		vIL-8 Prom	
JC5-002-a	MPF 57 P12 011103		plasmid	JCU		vIL-8 Prom	
JC5-002-b	MPF 57 P12 011103		plasmid	JCU		vIL-8 Prom	
JC5-002-c	MPF 57 P12 011103		plasmid	JCU		vIL-8 Prom	
JC5-002-d	MPF 57 P12 011103		plasmid	JCU		vIL-8 Prom	
JC5-003-a	MPF 57/B1 179-6 P7 200904		plasmid	JCU		vIL-8 Prom	
JC5-003-b	MPF 57/B1 179-6 P7 200904		plasmid	JCU		vIL-8 Prom	
JC5-003-c	MPF 57/B1 179-6 P7 200904		plasmid	JCU		vIL-8 Prom	
JC5-003-d	MPF 57/B1 179-6 P7 200904		plasmid	JCU		vIL-8 Prom	
JC5-004-a	MPF 57/B2 0340.200s-P5 13005		plasmid	JCU		vIL-8 Prom	
JC5-004-b	MPF 57/B2 0340.200s-P5 13005		plasmid	JCU		vIL-8 Prom	
JC5-004-c	MPF 57/B2 0340.200s-P5 13005		plasmid	JCU		vIL-8 Prom	
JC5-004-d	MPF 57/B2 0340.200s-P5 13005		plasmid	JCU		vIL-8 Prom	
JC5-005-a	MPF 57/B1 176/79-P3 270404		plasmid	JCU		vIL-8 Prom	
JC5-005-b	MPF 57/B1 176/79-P3 270404		plasmid	JCU		vIL-8 Prom	
JC5-005-c	MPF 57/B1 176/79-P3 270404		plasmid	JCU		vIL-8 Prom	
JC5-005-d	MPF 57/B1 176/79-P3 270404		plasmid	JCU		vIL-8 Prom	

original isolate name	alias name	amount of material held (ml)	storage method	storage location	Origin	DNA details	comments
JC5-007-a	MPF 57B2 W7B3 - P7 200904		plasmid	JCU		vIL-8 Prom	
JC5-007-b	MPF 57B2 W7B3 - P7 200904		plasmid	JCU		vIL-8 Prom	
JC5-007-c	MPF 57B2 W7B3 - P7 200904		plasmid	JCU		vIL-8 Prom	
JC5-007-d	MPF 57B2 W7B3 - P7 200904		plasmid	JCU		vIL-8 Prom	
JC5-008-a	FT 158 P6 030603		plasmid	JCU		vIL-8 Prom	
JC5-008-b	FT 158 P6 030603		plasmid	JCU		vIL-8 Prom	
JC5-008-c	FT 158 P6 030603		plasmid	JCU		vIL-8 Prom	
JC5-008-d	FT 158 P6 030603		plasmid	JCU		vIL-8 Prom	
JC5-009-a	179/2 P8 260904		plasmid	JCU		vIL-8 Prom	
JC5-009-b	179/2 P8 260904		plasmid	JCU		vIL-8 Prom	
JC5-009-c	179/2 P8 260904		plasmid	JCU		vIL-8 Prom	
JC5-009-d	179/2 P8 260904		plasmid	JCU		vIL-8 Prom	
JC5-010-a	179/3 P6 120904		plasmid	JCU		vIL-8 Prom	
JC5-010-b	179/3 P6 120904		plasmid	JCU		vIL-8 Prom	
JC5-010-c	179/3 P6 120904		plasmid	JCU		vIL-8 Prom	
JC5-010-d	179/3 P6 120904		plasmid	JCU		vIL-8 Prom	
JC5-011-a	179/8 P5		plasmid	JCU		vIL-8 Prom	
JC5-011-b	179/8 P5		plasmid	JCU		vIL-8 Prom	
JC5-011-c	179/8 P5		plasmid	JCU		vIL-8 Prom	
JC5-011-d	179/8 P5		plasmid	JCU		vIL-8 Prom	
JC5-012-a	192/8 P6 130305		plasmid	JCU		vIL-8 Prom	
JC5-012-b	192/8 P6 130305		plasmid	JCU		vIL-8 Prom	
JC5-012-c	192/8 P6 130305		plasmid	JCU		vIL-8 Prom	
JC5-012-d	192/8 P6 130305		plasmid	JCU		vIL-8 Prom	
JC5-013-a	199/9 P6 130305		plasmid	JCU		vIL-8 Prom	
JC5-013-b	199/9 P6 130305		plasmid	JCU		vIL-8 Prom	

original isolate name	alias name	amount of material held (ml)	storage method	storage location	Origin	DNA details	comments
JC5-013-c	199/9 P6 130305		plasmid	JCU		vIL-8 Prom	
JC5-013-d	199/9 P6 130305		plasmid	JCU		vIL-8 Prom	
JC5-014-a	132/5 P3 061003		plasmid	JCU		vIL-8 Prom	
JC5-014-b	132/5 P3 061003		plasmid	JCU		vIL-8 Prom	
JC5-014-c	132/5 P3 061003		plasmid	JCU		vIL-8 Prom	
JC5-014-d	132/5 P3 061003		plasmid	JCU		vIL-8 Prom	
JC5-015-a	MPF 57 P9 300801		plasmid	JCU		pp38 SNP	
JC5-015-b	MPF 57 P9 300801		plasmid	JCU		pp38 SNP	
JC5-015-c	MPF 57 P9 300801		plasmid	JCU		pp38 SNP	
JC5-015-d	MPF 57 P9 300801		plasmid	JCU		pp38 SNP	
JC5-016-a	MPF 57 P12 011103		plasmid	JCU		pp38 SNP	
JC5-016-b	MPF 57 P12 011103		plasmid	JCU		pp38 SNP	
JC5-016-c	MPF 57 P12 011103		plasmid	JCU		pp38 SNP	
JC5-016-d	MPF 57 P12 011103		plasmid	JCU		pp38 SNP	
JC5-017-a	MPF 57/B1 179-6 P7 200904		plasmid	JCU		pp38 SNP	
JC5-017-b	MPF 57/B1 179-6 P7 200904		plasmid	JCU		pp38 SNP	
JC5-017-c	MPF 57/B1 179-6 P7 200904		plasmid	JCU		pp38 SNP	
JC5-017-d	MPF 57/B1 179-6 P7 200904		plasmid	JCU		pp38 SNP	
JC5-018-a	MPF 57/B2 0340.200s-P5 13005		plasmid	JCU		pp38 SNP	
JC5-018-b	MPF 57/B2 0340.200s-P5 13005		plasmid	JCU		pp38 SNP	
JC5-018-c	MPF 57/B2 0340.200s-P5 13005		plasmid	JCU		pp38 SNP	
JC5-018-d	MPF 57/B2 0340.200s-P5 13005		plasmid	JCU		pp38 SNP	
JC5-019-a	MPF 57/B1 176/79-P3 270404		plasmid	JCU		pp38 SNP	
JC5-019-b	MPF 57/B1 176/79-P3 270404		plasmid	JCU		pp38 SNP	
JC5-019-c	MPF 57/B1 176/79-P3 270404		plasmid	JCU		pp38 SNP	
JC5-019-d	MPF 57/B1 176/79-P3 270404		plasmid	JCU		pp38 SNP	

original isolate name	alias name	amount of material held (ml)	storage method	storage location	Origin	DNA details	comments
JC5-022-a	FT 158 P6 030603		plasmid	JCU		pp38 SNP	
JC5-022-b	FT 158 P6 030603		plasmid	JCU		pp38 SNP	
JC5-022-c	FT 158 P6 030603		plasmid	JCU		pp38 SNP	
JC5-022-d	FT 158 P6 030603		plasmid	JCU		pp38 SNP	
JC5-023-a	179/2 P8 260904		plasmid	JCU		pp38 SNP	
JC5-023-b	179/2 P8 260904		plasmid	JCU		pp38 SNP	
JC5-023-c	179/2 P8 260904		plasmid	JCU		pp38 SNP	
JC5-023-d	179/2 P8 260904		plasmid	JCU		pp38 SNP	
JC5-024-a	179/3 P6 120904		plasmid	JCU		pp38 SNP	
JC5-024-b	179/3 P6 120904		plasmid	JCU		pp38 SNP	
JC5-024-c	179/3 P6 120904		plasmid	JCU		pp38 SNP	
JC5-024-d	179/3 P6 120904		plasmid	JCU		pp38 SNP	
JC5-025-a	179/8 P5		plasmid	JCU		pp38 SNP	
JC5-025-b	179/8 P5		plasmid	JCU		pp38 SNP	
JC5-025-c	179/8 P5		plasmid	JCU		pp38 SNP	
JC5-025-d	179/8 P5		plasmid	JCU		pp38 SNP	
JC5-026-a	192/8 P6 130305		plasmid	JCU		pp38 SNP	
JC5-026-b	192/8 P6 130305		plasmid	JCU		pp38 SNP	
JC5-026-c	192/8 P6 130305		plasmid	JCU		pp38 SNP	
JC5-026-d	192/8 P6 130305		plasmid	JCU		pp38 SNP	
JC5-027-a	199/9 P6 130305		plasmid	JCU		pp38 SNP	
JC5-027-b	199/9 P6 130305		plasmid	JCU		pp38 SNP	
JC5-027-c	199/9 P6 130305		plasmid	JCU		pp38 SNP	
JC5-027-d	199/9 P6 130305		plasmid	JCU		pp38 SNP	
JC5-028-a	132/5 P3 061003		plasmid	JCU		pp38 SNP	
JC5-028-b	132/5 P3 061003		plasmid	JCU		pp38 SNP	

original isolate name	alias name	amount of material held (ml)	storage method	storage location	Origin	DNA details	comments
JC5-028-c	132/5 P3 061003		plasmid	JCU		pp38 SNP	
JC5-028-d	132/5 P3 061003		plasmid	JCU		pp38 SNP	
JC5-029-a	BH16 low pass		plasmid	JCU		vIL-8 Prom	
JC5-029-b	BH16 low pass		plasmid	JCU		vIL-8 Prom	
JC5-029-c	BH16 low pass		plasmid	JCU		vIL-8 Prom	
JC5-029-d	BH16 low pass		plasmid	JCU		vIL-8 Prom	
JC5-030-a	BH16 vaccine		plasmid	JCU		vIL-8 Prom	
JC5-030-b	BH16 vaccine		plasmid	JCU		vIL-8 Prom	
JC5-030-c	BH16 vaccine		plasmid	JCU		vIL-8 Prom	
JC5-030-d	BH16 vaccine		plasmid	JCU		vIL-8 Prom	
JC5-031-a	Woodlands #1 P14		plasmid	JCU		vIL-8 Prom	
JC5-031-b	Woodlands #1 P14		plasmid	JCU		vIL-8 Prom	
JC5-031-c	Woodlands #1 P14		plasmid	JCU		vIL-8 Prom	
JC5-031-d	Woodlands #1 P14		plasmid	JCU		vIL-8 Prom	
JC5-032-a	Woodlands #1 High pass		plasmid	JCU		vIL-8 Prom	
JC5-032-b	Woodlands #1 High pass		plasmid	JCU		vIL-8 Prom	
JC5-032-c	Woodlands #1 High pass		plasmid	JCU		vIL-8 Prom	
JC5-032-d	Woodlands #1 High pass		plasmid	JCU		vIL-8 Prom	
JC5-033-a	CVI 988 vaccine		plasmid	JCU		vIL-8 Prom	
JC5-033-b	CVI 988 vaccine		plasmid	JCU		vIL-8 Prom	
JC5-033-c	CVI 988 vaccine		plasmid	JCU		vIL-8 Prom	
JC5-033-d	CVI 988 vaccine		plasmid	JCU		vIL-8 Prom	
JC5-034-a	BH16 low pass		plasmid	JCU		pp38 SNP	
JC5-034-b	BH16 low pass		plasmid	JCU		pp38 SNP	
JC5-034-c	BH16 low pass		plasmid	JCU		pp38 SNP	
JC5-034-d	BH16 low pass		plasmid	JCU		pp38 SNP	

original isolate name	alias name	amount of material held (ml)	storage method	storage location	Origin	DNA details	comments
JC5-035-a	BH16 vaccine		plasmid	JCU		pp38 SNP	
JC5-035-b	BH16 vaccine		plasmid	JCU		pp38 SNP	
JC5-035-c	BH16 vaccine		plasmid	JCU		pp38 SNP	
JC5-035-d	BH16 vaccine		plasmid	JCU		pp38 SNP	
JC5-036-a	Woodlands #1 P14		plasmid	JCU		pp38 SNP	
JC5-036-b	Woodlands #1 P14		plasmid	JCU		pp38 SNP	
JC5-036-c	Woodlands #1 P14		plasmid	JCU		pp38 SNP	
JC5-036-d	Woodlands #1 P14		plasmid	JCU		pp38 SNP	
JC5-037-a	Woodlands #1 High pass		plasmid	JCU		pp38 SNP	
JC5-037-b	Woodlands #1 High pass		plasmid	JCU		pp38 SNP	
JC5-037-c	Woodlands #1 High pass		plasmid	JCU		pp38 SNP	
JC5-037-d	Woodlands #1 High pass		plasmid	JCU		pp38 SNP	
JC5-038-a	CVI 988 vaccine		plasmid	JCU		pp38 SNP	
JC5-038-b	CVI 988 vaccine		plasmid	JCU		pp38 SNP	
JC5-038-c	CVI 988 vaccine		plasmid	JCU		pp38 SNP	
JC5-038-d	CVI 988 vaccine		plasmid	JCU		pp38 SNP	
JC5-039-a	FC126 HVT		plasmid	JCU		qPCR prod	
JC5-039-b	FC126 HVT		plasmid	JCU		qPCR prod	
JC5-039-c	FC126 HVT		plasmid	JCU		qPCR prod	
JC5-039-d	FC126 HVT		plasmid	JCU		qPCR prod	
JC5-040-a	GAPDH Avian		plasmid	JCU		qPCR prod	
JC5-040-b	GAPDH Avian		plasmid	JCU		qPCR prod	
JC5-040-c	GAPDH Avian		plasmid	JCU		qPCR prod	
JC5-040-d	GAPDH Avian		plasmid	JCU		qPCR prod	

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Plain English Compendium Summary

Project Title:	Isolation and titration of selected avian pathogens in cell culture
Project No.:	09-26
Researcher:	Dr. Katrin Renz
Organisation:	University of New England
Phone:	02-6773 3008
Fax:	02-6773 3922
Email:	krenz@une.edu.au
Objectives	• To audit the current stock materials of MDV isolates in Australia • To grow one or more MDV isolates to a high titre (>10^4 pfu/ml) in CEF cell culture. • To analyse DNA from cell cultured virus material for viral copy numbers in order to relate them with plaque counts. • To cryopreserve cell-culture derived infective and titrated material for long-term storage and future use.
Background	Recent research projects RIRDC UNE-83J and Poultry CRC 03-17 aimed at defining and monitoring the virulence of MDV strains in Australia and the level of vaccinal protection afforded by current vaccines encountered an unexpected problem in the extreme difficulty in growing Australian MDV strains to moderate titre (>10^4pfu/ml) so that the isolates could be tested *in vivo*. Earlier Australian researchers have also reported significant problems growing MDV to high titre. On the other hand several groups around the world routinely grow field MDV isolates to titres of 10^6-10^9 pfu/ml. In late 2007 Prof Walkden- Brown joined Dr Nair at IAH-C, UK, on a 10-month sabbatical for which he was awarded a BBSRC Underwood Fellowship. The IAH-C is the OIE international reference laboratory for MD and routinely isolates Marek's disease virus on cell culture. Dr Katrin Renz visited Dr Nair's group at Compton in May 2008 and could familiarize herself with the cell culture methods used at IAH-C. This project aimed to deliver improved methodologies for both MDV isolation and propagation in cell culture based on best international practice. This will significantly improve the capacity for the Australian poultry industry to manage one of its most important endemic diseases.
Research	Nine different MDV isolates were attempted to grow on chicken embryo fibroblast (CEF) cell cultures with the aim to achieve a high titre (>10^4pfu/ml) within the lowest possible number of passages. At passage number 3-4, the isolates were titrated on CEF. After enumeration of plaques under an inverted microscope, the titre was calculated and the virus isolates cryopreserved and stored in liquid nitrogen for future use.
Outcomes	Five of the nine MDV isolates grew to titres ranging from 60,000-165,000 pfu/ml. The number of passages until the virus was titrated and cryopreserved was 3 for the isolate Woodlands1 for which the original infective material was derived from a fresh spleen from an unvaccinated, specific pathogen free chicken. The other 4 isolates were titrated and cryopreserved at passage number 4. The original infective material for these isolates was derived from low passage, low titre cell cultured material which had been stored in liquid nitrogen. The remaining four isolates did not show any plaques after the 3rd

	passage and were discarded. The original material from one of these isolates (MPF199) was obviously contaminated as the CEF cell layer disassembled within 24 hours of infection with this isolate.
Implications	This project delivered improved methodologies for MDV isolation and propagation in cell culture based on best international practice. This significantly improves the capacity for the Australian poultry industry to manage one of its most important endemic diseases. The work of this project could serve as a template for future isolation and propagation of MDV in cell culture. The major implications of this project are: 1. Australian MDV isolates can be grown in cell culture to high titres ($\geq 10^4$ pfu/ml) within low passage numbers (3-4), especially when fresh materials, i.e. splenocytes are used. 2. The cell culture media, serum and cell type significantly influences the ability to successfully isolate and propagate MDV isolates. Chicken embryo fibroblasts are easier to prepare, handle and grow and were therefore chosen for all work done in this project. This is in accordance with the IAH institute in Compton, UK, which is the international reference laboratory for Marek's disease. 3. Results from qPCR are not necessarily consistant with plaque counts. The reason might be that PCR will amplify both dead, damaged and live virus particles whereas visible plaques in cell culture only represent live virus.
Publications	Dr. Katrin Renz is expected to write a short journal paper on the work arising from this project.